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D. E. SALMON, D. V. M., Chief of Bureau.

INVISIBLE MICROORGANISMS.

BY

M. DORSET, M. D.,
Assistant Chief of Biochemic Division, Bureau of Animal Industry.

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By M. Dorset, M. D.,
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A REVIEW OF THE SITUATION.

Fifty years ago the nature of infectious diseases was just beginning to be understood. The then existent beliefs concerning the origin of infectious diseases rested almost entirely upon a theoretical basis, and it was not until the beginning of the era of experimental medicine, in which movement Pasteur was the great leader, that any headway was made in the solution of those problems which were finally so brilliantly worked out by Pasteur and his collaborators of that period. The existence of bacteria and other microscopic forms of life had been recognized for more than a century when, in 1850, Pollender and Davaine made their observations upon anthrax, their work being the first experimental evidence of the fact that certain forms of bacteria occupied a causal relation to particular infectious diseases.

During the period between 1850 and 1875 the controversy regarding the spontaneous generation of microorganisms was the all-absorbing topic among biologists, and although this debate did not include the etiology of infectious diseases it involved questions, such as the cause of fermentation, which were so closely allied that the methods employed in the study of the one were in great part applicable to the other.

Thus it was that the foundations of our prese t-day bacteriological technique were laid, and when, in 1881, Koch gave to the workers in bacteriology his solid-culture media and his "plate method," the principles of sterilization and the methods of staining were already well understood. It is no wonder, therefore, that the decade following Koch's announcement of the "plate method" of isolating bacteria in pure culture was notable for the great advances made in experimental medicine, and especially in that branch of it which concerns the etiology of infectious diseases.

Notwithstanding the many notable advances in that period, however, the causative agents of a certain number of human and animal diseases completely escaped discovery. New and supposedly favorable culture media were prepared; microscopes were improved in every detail of construction; new staining methods were devised; and, in fact, everything was done which might aid in determining the infectious agents in such diseases as measles, scarlatina, smallpox, rabies, bovine pleuro-

pneumonia, foot-and-mouth disease, rinderpest, and others of almost equal importance, and yet, as has just been stated, even at the present time we are almost entirely in the dark with regard to the etiology of the above-mentioned diseases.

In the year 1898, however, a number of important observations were made which were of such a nature that they seemed to give us a glimpse of an unknown world of infinitesimally small creatures whose existence had previously only been surmised.

It is with the idea of describing somewhat briefly the original discoveries and those which have followed them that this paper is written.

FOOT-AND-MOUTH DISEASE.

Foot-and-mouth disease is one of the most contagious maladies known to veterinary science, and has caused enormous losses to stock owners in Europe. Primarily it affects cattle, though few, if any, of the domestic animals are exempt, and is characterized by fever, loss of appetite, and emaciation, the last two symptoms being in large part due to the development of vesicles in the mouth, which cause swelling and tenderness of the buccal mucous membrane. Vesicles appear on the udders of cows and on the skin surrounding the hoofs. The mortality from this disease among affected cattle probably averages 1 or 2 per cent, though it may be very much greater. The chief loss, however, is to be noted in the general condition of the herd, which is, as a rule, 30 per cent poorer than before the attack of the disease.

In 1898 Loeffler and Frosch presented to the German Government a report of the results which they, as a commission, had obtained from their investigations of foot-and-mouth disease. They showed that the fluid contained in the buccal vesicles of affected cattle was extremely infectious, and that a very small amount of such material, when in a fresh state, sufficed to bring about typical attacks of foot-and-mouth disease in cattle and hogs, and this notwithstanding the fact that they were unable, even after the most careful search, to ascertain the existence of any microorganism whatever in certain lots of this fluid, either by staining or by culture experiments. Knowing, however, by experience that the infectious agent was present, and being desirous of obtaining some method of producing immunity, they conceived the idea that the filtered lymph from an animal in the acute stage of foot-and-mouth disease might be used for this purpose with advantage, the object of the filtration being to separate all the corpuscular elements from the soluble portion, the latter to be used for the production of immunity. With this idea in view, therefore, a certain quantity of virulent lymph, to which was added 39 parts of water, was passed repeatedly through a filter (Berkefeld) of infusorial earth. Before the filtration, however, a considerable amount of a culture of *Bacil-*

lus fluorescens was added to the diluted virus in order that that bacillus might serve as a check on the efficacy of the filter. The filtrate was repeatedly tested, but at no time did *B. fluorescens* or any other microorganism which could be recognized by cultural methods appear.

A number of calves were next inoculated with quantities of the filtrate which were the equivalents of from one-tenth to one-fortieth cubic centimeters of the original lymph. To the very great surprise of the experimenters, the calves that were inoculated with the filtered lymph sickened in the same length of time as did the control animals, which received a corresponding amount of unfiltered lymph, and, in addition, they exhibited all the typical symptoms of foot-and-mouth disease, such as high fever and vesicles in the mouth and on the feet.

In order to confirm, if possible, this very unexpected occurrence, the experiments were repeated a number of times on calves and hogs, and always with the same result when fresh lymph was used. Here, then, was possibly a new fact for bacteriologists to reckon with, for Loeffler and Frosch had in their filtrate either an extremely powerful toxin or an organism which passed regularly through Berkefeld filters, which were fine enough to hold back *B. fluorescens*.

In order to show that they were not dealing with a toxin, Loeffler and Frosch compared the disease-producing power of their lymph with the most powerful tetanus toxin that had ever been reported, and found, according to calculations based upon the body weight of the animals and the amount of virus used, and taking into consideration the transference of the disease from an animal treated with filtered lymph to others, that if the disease produced by the filtered lymph was due to a toxin it was by far the most powerful ever known, its toxic value being 1:2,500,000,000,000. Such an extremely toxic substance is inconceivable, and, besides, we know of no disease which may be induced by one-fiftieth cubic centimeters of toxin and then be carried successively through six animals, using continually the same minute dose. It would therefore seem to be reasonably certain from the original experiments of Loeffler and Frosch that they had a living micrcorganism in their filtrates, and that this microorganism passed through the Berkefeld filter. More recently Loeffler has stated that, while the virus of foot-and-mouth disease passes through Berkefeld filters, it is retained by the finer-pored Kitasato filter, thus definitely disposing of the idea that the infecting substance may be in solution.

BOVINE PLEURO-PNEUMONIA.

Within a very short while after the publication of the researches of Loeffler and Frosch, the paper of Nocard and Roux upon the etiology of bovine pleuro-pneumonia appeared. The work of these French savants, done in collaboration with Borrel, Salimbeni, and Dujardin-

Beaumetz, was as remarkable in its way as was the work of their German colleagues, for they not only brought forward an entirely new principle in the cultivation of microorganisms, but by means of this new method they were able to demonstrate positively the existence of a microorganism, which is certainly on the very border line which separates the visible from the invisible—the microscopic from the ultramicroscopic.

Bovine pleuro-pneumonia is an infectious disease of cattle which, by reason of its infectiousness and virulence, has been a source of great loss to cattle owners wherever it has existed. The disease is characterized especially by a serous exudate into the pulmonary interlobular tissue. It is quite infectious, and the mortality produced by it is considerably greater than that of foot-and-mouth disease. It is easily transmitted experimentally from one animal to another by subcutaneous inoculation. Where such an inoculation of a susceptible animal is made great swelling of the surrounding tissue follows, and this is accompanied by fever. In this subcutaneous effusion it is impossible to see any microorganisms, and cultures made from it in the usual way remain sterile.

The collodion-sac method of cultivating microorganisms *in vivo*, which was originated by Metchnikoff, has been most successfully employed by these authors in the following way: Thin sacs of collodion containing sterile nutritive bouillon were inoculated with a minute quantity of the serous effusion of bovine pleuro-pneumonia. These sacs, after being hermetically sealed, were introduced into the peritoneal cavity of rabbits. Fifteen or twenty days later the rabbits were killed and the unbroken sacs removed. The bouillon contained in them, instead of being perfectly clear, as it had been at the time it was introduced, was found to be opalescent. A collodion sac containing the same bouillon not inoculated was put into another rabbit at the same time and remained clear. When the above-mentioned opalescent culture fluid was examined microscopically with a magnification of 2,000 diameters and with an extremely powerful illumination, numberless extremely minute motile points could be made out. These remarkably small bodies are now generally conceded to be the cause of bovine pleuro-pneumonia. They are of such very small size that it has never been possible to determine definitely anything in regard to their form, even after they have been stained.

As proof that they were dealing with a living virus, Nocard and Roux could rely upon the motility of the bodies which they found, and also more firmly upon the fact that they were able to propagate their organism in successive collodion-sac cultures, and finally by the use of specially prepared culture media they were able to obtain and transfer indefinitely cultures *in vitro*, and with these cultures they produced typical attacks of pleuro-pneumonia in cattle.

It is very interesting to note the effect of their collodion-sac cultures

upon the rabbits. Those rabbits which received the sacs inoculated with pleuro-pneumonia became emaciated, some of them even dying on the fifteenth or twentieth day. Those rabbits which received uninoculated sacs of bouillon remained perfectly well. As rabbits are entirely refractory to inoculations with the virus of pleuro-pneumonia made in the ordinary way, this experiment seems to show that they may be susceptible to the soluble toxins generated by this microorganism, although they are capable of overcoming the living virus, provided it is not protected in some such way as by the collodion sac. In other words, we have in the behavior of the rabbit toward pleuropneumonia an instance of an animal which is immune from the body of the microorganism, but susceptible to its toxins.

In a paper published about a year later Roux and Nocard announced that they had been able to filter the diluted pleuro-pneumonia virus through a Berkefeld, and also through a Chamberland F cylinder, but were unsuccessful in their attempts to filter it through a Chamberland B, thus indicating that an organism need not necessarily be ultramicroscopic because it will traverse with regularity a Chamberland filter and a Berkefeld filter, they having previously shown by their successful cultures that the pleuro-pneumonia virus is just within the limits of visibility.

Since the researches upon pleuro-pneumonia and foot-and-mouth disease were published there have been many efforts to apply the principles there introduced to other diseases which had baffled all previous attempts to determine their cause. The majority of such investigations dealt with animal diseases, for the reason that they could be reproduced experimentally when it was desired to do so.

YELLOW FEVER.

First of all, concerning yellow fever, mention should be made of the epoch-making experiments of Reed, Carroll, and their associates. These investigators had already shown by a series of daring and conclusive experiments that yellow fever is transmitted only through the bite of a mosquito (genus *Stegomyia*), when, a little later, they announced the results of some filtration experiments with a Berkefeld laboratory cylinder. A certain amount of blood was drawn from the elbow vein of a man who was suffering from a mild attack of yellow fever. The serum which separated from the clot was diluted with an equal volume of sterile distilled water and filtered slowly through a Berkefeld laboratory filter which had been previously sterilized. The clot which remained was beaten up with distilled water. One nonimmune man inoculated with 0.75 c. c. of the aqueous extract of this clot developed a mild but typical attack of yellow fever. Three nonimmunes inoculated with double the amount of the same material, except that it had been previously heated to 55° C. for ten minutes, remained perfectly well. These two experiments showed that the

blood which was being used contained the true cause of yellow fever, and also that the virus, whatever its nature, was destroyed by an exposure for ten minutes to a temperature of 55° C. The filtered serum mentioned above, and which was derived from the same blood, was used for the inoculation of three other nonimmunes, an equivalent of 1.5 c. c. of the original serum being used for each inoculation. Two of these nonimmunes developed typical attacks of mild yellow fever as a result of these inoculations with filtered serum; the third one remained well. This nonimmune, who resisted the first inoculation as just noted, was subsequently inoculated with 1.5 c. c. of blood taken from one of the cases of yellow fever produced by filtered serum. As a result of the second inoculation he became typically ill with yellow fever. From the foregoing experiments, which, on account of their great importance, have been given in considerable detail, Reed and Carroll deduced the following conclusions concerning yellow fever: (1) The cause of the disease exists in the blood; (2) its vitality is destroyed by exposure for ten minutes to a temperature of 55° C.; (3) the virus is of extremely small size, as is shown by its passage through a Berkefeld filter.

As proofs that they had in their filtrates a living virus which was capable of multiplication, they quote their experiments showing that the thermal death point of the yellow-fever contagium is 55° C. for ten minutes. No known toxin will be destroyed by such an exposure as this. As proof that they were dealing with a virus which multiplied, they have the inoculation of the last nonimmune with blood from another who had been inoculated with filtered serum. Immediately after the filtration of the yellow-fever blood the Berkefeld filter which had been used was resterilized by steam and its effectiveness tested with cultures of *Staphylococcus pyogenes aureus*, and it proved to be entirely impervious to the above-named organism. The work of these American investigators has recently been entirely confirmed by the French yellow-fever commission, consisting of MM. Marchoux, Salimbeni, and Simond, who also conducted filtration experiments and found that in the serum from a yellow-fever patient the virus traverses a Chamberland F filter without dilution; the Chamberland B, under the same conditions, retained the virus. The microbe of yellow fever, being able to traverse a Chamberland F filter without dilution of the albuminous fluid in which it is contained, is probably of ultramicroscopic size. At least it seems likely that there are some forms of that parasite, as it exists in the human blood, which are too minute to be recognized even by our most powerful microscopes.

HORSE SICKNESS.

"Horse sickness," a South African disease of horses, is unknown in other parts of the world, although it has caused great destruction in that section of Africa. Horses chiefly are affected, though mules and

asses also contract the disease. The latter animals suffer from a milder form, and a few passages through them serve to weaken materially the potency of the virus. Summer is the time that the disease appears, and then chiefly in the low-lying districts, where the vegetation is abundant and the dews are heavy. Those animals that are allowed to run at large at night are especially liable to be attacked, while others of the same lot that are confined in stables remain well. The disease is not contagious, but may be transferred from one animal to another by subcutaneous inoculation of diseased blood. All of these peculiarities tend to indicate that the disease is induced by the bite of an insect, and probably a nocturnal one. The malady is usually described as existing in two forms—the lung sickness (dunpaardziekte) and the head sickness (dikkopziekte). Both forms are characterized by extensive serous exudations, an excess of such exudations in particular parts of the body being sufficient to classify the attack as of one form or the other. The attack usually begins with a moderate fever which shows an evening exacerbation. The lung symptoms develop very rapidly; there is difficult breathing, with the discharge of a faintly reddish tinged froth from the mouth and nostrils. Death appears to take place as a result of the plugging of the air passages by the extensive serous exudate. The form known as dikkopziekte, while exhibiting to a certain degree the lung symptoms, is characterized especially by extensive swelling of the head and neck. At the autopsy, in the pulmonary form, the lung tissue is found to be engorged with a serous exudate, and in the head sickness the subcutaneous tissues of the head and neck are distended with an exudate of a like character.

Most of the pioneer work concerning the etiology of horse sickness was done by Edington, who finally decided that it was caused by a mold which he found in the blood of all horses dying of this disease. Edington's idea, however, has very recently fallen into disfavor on account of the work of McFadyean and Nocard, whose experiments appear to place this disease also in the class with those caused by "invisible" microorganisms. McFadyean's experiments related chiefly to the filterability of the virus. He used a vacuum which varied from 26 to 29 inches and was applied to different filters. His filtrations proceeded slowly. In one instance, where serum was diluted with four volumes of water, twenty-two hours were required for the passage of 6 ounces of fluid.[a] McFadyean succeeded in passing the virus of horse sickness through a Berkefeld filter three times, through a Chamberland F four times, and through a Chamberland B one time. In all cases the filtered virus produced typical attacks of the disease in horses, but in no instance was he able to obtain a culture from these filtrates, they hav-

[a] There was here the possibility of microorganisms having grown through the filter.

ing always remained sterile. Since he succeeded in passing the virus through the Chamberland B cylinder, McFadyean believed the organism of horse sickness to be the smallest yet described.

Nocard does not agree with McFadyean in all respects. He describes one experiment in which he was able to filter the virus of horse sickness through a Berkefeld filter when the exudate was diluted in the proportion of 1 to 33 with river water. This filtrate produced typical attacks of the disease in two horses, although cultures from the filtrate showed no growth whatever. Nocard did not succeed in passing the virus through either the Chamberland F or B cylinders. Nocard states that he made many efforts to cultivate the microorganism of this disease, but all of them failed. He used liquid and solid media of many different kinds; cultures were made in vacuo, in the presence of oxygen or of inert gases; the collodion sac, with the use of which Nocard was of course perfectly familiar, was tried as a last resort, and was placed in the peritoneal cavity of many different species of animals, but always with the same result—the supposed microorganism refused to grow. One interesting fact brought out by Nocard is that virulent blood from a case of horse sickness may be sealed in tubes and kept in the dark at room temperature for more than two years without losing its infectiousness.

It will be agreed that in horse sickness we have a disease caused by a filterable virus. Its detailed characteristics have not as yet been made out.

BIRD PEST.

There is in Europe a highly infectious and fatal disease of chickens which has never been reported in this country, although it seems to be gaining quite a foothold abroad. It is generally admitted that the disease in question has existed in Italy for more than ten years, and the researches of Lode and Gruber and of Dubois show that it has more recently appeared in Belgium and in the Tyrolean Alps. There is much confusion in regard to the name for this disease, the terms "Vogel-pest," "Hühner-pest," "exudative typhus," and "*Kyanolophiea gallinarum*" having all been used by one or more writers. The name Vogel-pest or bird pest, as proposed by Centanni, seems to be sufficiently distinctive for our purpose, and it will be used in this paper.

It is to Centanni that the credit must be given for the first complete description of this disease and for the first description of the nature of the virus by which it is caused. This disease, which, according to Centanni, has been recognized as a distinct type for more than ten years, resembles chicken cholera, but does not correspond to it in all particulars. The symptoms of the disease may be summed up as follows: The chicken on the first day of illness appears to be somewhat unwell; on the second day it has ceased eating, the feathers

are ruffled, and the comb is darker in color; on the third day it is found dead or dying. The above is the usual course of the disease, but there may be instances in which the animal will live only a few hours, or, on the contrary, it may survive a week or more. The temperature just before death falls to 30° C. or less.

At the autopsy evidences of pericarditis are usually present, and there may be ecchymoses on the surface of the heart. There is usually some pleuritis. The lungs are more or less congested, this even extending at times to a pneumonic consolidation. The liver is enlarged and the spleen and kidneys are hyperemic. The intestinal lesions are never so severe as is seen in fowl cholera. Not all of the above lesions are, as a rule, found at one autopsy; indeed, in some instances nothing abnormal can be seen except a few congested areas scattered here and there in the various organs. The heart blood or aqueous extract of the lungs or liver almost invariably brings on an attack of the disease when a minute quantity is injected subcutaneously or when fed. Maggiora and Valenti found, after a large number of experiments, that 4 c. c. of a solution of virulent blood in which the blood was present in a proportion of 1:125,000,000, would destroy a young hen of 370 grams weight, and they intimate that, although they succeeded in causing the death of hens with such an extremely minute quantity of the virus, as noted above, this mark may be passed, and that no doubt blood will be found which will kill in even smaller doses. There is some variation in opinion in regard to the infectiousness of this disease for other fowls. Pigeons succumb to subcutaneous injections of the blood of diseased chickens, and by some authors there is said to be an unusual mortality among the wild birds in neighborhoods where the disease is prevailing. Guinea pigs and mice are not susceptible to the infection. Centanni regards rabbits as being subject to a kind of toxemia as a result of inoculation with large quantities of the virus. The infectious agent of this disease is very easily destroyed by heat, light, or drying. Maggiora and Valenti found that virulent blood allowed to dry spontaneously and kept in the dark for twenty-two days was still virulent, but in forty-two days its virulence had disappeared. In diffuse light the virulence was retained for fifteen days; in very bright daylight the virulence was lost in forty hours. The virus was destroyed by an exposure of one-half hour at 60° C.

Notwithstanding the above-described excessive virulence of the blood and body juices of chickens attacked by bird-pest, it has been impossible to isolate in pure culture any organism that is capable of producing this very destructive disease. Frequently the culture tubes remain perfectly sterile when the blood from which they were made is proved to be highly virulent. Cultures of a number of different organisms have been isolated by the several investigators, but each one has been finally proved to be only a contamination or a secondary

invader. The collodion-sac method was tried by Centanni, but without success. All attempts to discover the infectious agent in the blood and tissues by various methods of staining have proved futile. We are brought, then, to face another disease which seems to be due to an invisible microorganism.

Centanni and Maggiora and Valenti found that the virus retained its virulence after dilution and passage through a Berkefeld or a Chamberland F filter. According to Maggiora and Valenti, it will not pass through a Chamberland K. Dubois found that the virus passed through Chamberland filters, but the particular grade of filter is not mentioned. Lode and Gruber succeeded in passing the virus through Berkefeld filters only. Several authors record the passage of the filtered virus through four or more hens, successively, thus showing that the virus in the filtrate is capable of multiplication.

SHEEP POX.

There are a number of synonyms for this disease, the most important being clavelée and ovine variola. Sheep pox, in its general characters, resembles the smallpox of the human subject and is characterized by an eruption of small pustules, which may cover the entire cutaneous surface of the body. When infection of a flock takes place the disease spreads by regular stages called commonly the "moons." This is explained by the fact that a period of from twenty to twenty-five days is required from the time of infection until the infected animal becomes capable of communicating it to others,[a] and the moon, of course, has nothing whatever to do with the periodical advances of the disease in infected herds. In addition to goats and sheep, the disease may be communicated to dogs, pigs, horses, and cattle by inoculation. The virus is of great vitality and, when dry and protected from the action of light and air, will remain virulent an indefinite time. This explains the danger in sheepskins and wool. Any of the strong antiseptics suffice to destroy the virus. The disease has existed from time immemorial in the Mediterranean countries—Spain, Italy, the south of France, Algeria, and Tunis—all having the disease present in endemic form. Before the researches of Borrel, nothing very definite was known as to its nature. His first investigations consisted chiefly of filtration experiments. Material taken from a pustule and suspended in water was virulent even after an enormous dilution. Filtration of such diluted virus through a Berkefeld cylinder gave a filtrate which was sterile in so far as a visible growth of microorganisms was concerned, but which was still endowed with all the disease-producing power of the unfiltered material. Borrel then employed Chamberland filters of varying porosity with the following results. When the

[a] Borrel. Annal. de l'Inst. Pasteur, 1903. No. 11, Vol. XVII.

filtration takes place rapidly under pressure, the virus of sheep pox passes through a Berkefeld cylinder sometimes, but never through a Chamberland F. The Chamberland F_4, F_5, and so on to F_{10}, allow it to pass through always. The graded Chamberland filters were numbered F_2, F_3, F_4, F_5, etc., to F_{10}. The accompanying figures indicate that under identical conditions the filters so marked have a rate of discharge two, three, or, at the highest, ten times that of the ordinary Chamberland F. In all cases when cultures were made from the filtrate and incubated at 37° C. they remained sterile. If the dilutions are made with ordinary tap water, a certain number of very minute vibrios pass through the filter and develop at 20° C. In order to obtain the virus of sheep pox in a pure state it is sufficient to use boiled water in making dilutions. The filtrate will retain its virulence for a long time. Borrel considers that his researches show that the microbe of sheep pox is ultramicroscopic and that the cellular inclusions described as parasites of vaccinia, of variola, of clavelée can not be the true cause of the disease, and that researches upon smallpox and other allied diseases must be directed to the study of microorganisms, like those which cause peripneumonia and foot-and-mouth disease.

RINDERPEST.

This most fatal of all cattle diseases had its origin in Asia, though for centuries it has periodically ravaged Europe, and has also invaded Africa. Practically all ruminants are susceptible, but none contract the disease so readily as cattle, and cattle are also the chief agents in spreading the infection. Pigs show a slight susceptibility. The horse, dog, bird, and rabbit are immune, and man also. The death rate in cattle which have had no means of acquiring resistance to the disease is usually from 80 to 95 per cent. The disease is characterized by a high temperature and by lesions which are most extensive in the mucous membrane of the intestinal tract. The blood of infected cattle may be used to bring about an attack in another animal. Animals which have recovered from an attack of the disease are immune from a second attack. The cause of this disease has never been discovered, though many organisms have been isolated which were believed for a time to be the true etiological agent. Semmer attributes the disease to certain minute bodies, which he believes are usually contained within certain cell nuclei, and Nicolle and Adil Bey agree with Semmer to a certain degree. The two authors last named have made the most recent and apparently the most important contributions to our knowledge concerning the etiology of rinderpest. Certain lots of blood which they knew to be infectious failed to give any cultures, nor could the authors by the most careful microscopic examination find any bacteria or other microorganisms in the infec-

tious material. The next step, therefore, was to pass the diluted virus through filters of porcelain and of infusorial earth. Three grades of filters were used: (1) Thin-walled Berkefeld, yet thick enough to retain any of the ordinary bacteria; (2) the ordinary Berkefeld; and (3) the Chamberland filter (marked "F"). Using the thin-walled Berkefeld, they obtained filtrates which were sterile (in the ordinary sense), but which sometimes killed the cattle inoculated with them, sometimes produced an immunity without causing visible illness, or, again, they might be inactive. With the ordinary Berkefeld filters the results were the same, except that negative experiments were more frequent than in the first set. With the Chamberland F cylinder successful filtration is rare, death never being produced, and only occasionally the filtrate confers immunity upon the inoculated animal. From the experiments given in detail by Nicolle and Adil Bey there can be no doubt that the virus of rinderpest is filterable under certain conditions, and the failure of certain of their experiments is explained by the authors with the supposition that the virus is situated normally within the leucocytes. When a highly virulent filtrate is obtained they suppose that a sufficient number of the minute bodies which are probably the exciting cause in rinderpest have become detached from the cells in which they dwell, and are thus free to pass through the filter pores. In the instances where the filtrates had a protective action enough of the specific microorganisms were allowed to pass for the production of immunity, but not enough to bring on an attack of the disease. The work of Nicolle and Adil Bey has not yet been confirmed, but until it has been successfully contradicted we must be inclined to believe that rinderpest, the great cattle scourge, is caused by a microorganism too small to be demonstrated by the means now at our command.

RABIES.

The cause of this disease, which was investigated extensively by Pasteur himself and later by some of his most distinguished pupils, remains unknown to the present day. Pasteur expressed the opinion in 1882 that the virus of rabies is ultramicroscopic, but only within the last year has anything been done to substantiate this hypothesis. Remlinger and Riffat Bey in two communications to the Societé de Biologie in Paris record the passage of the virus of rabies through a Berkefeld filter marked "V." The passage through this cylinder was not constant. In one instance two out of nine rabbits inoculated with the filtrate, in another instance four out of ten, and in another two out of seven died of typical rabies, and in every instance where death resulted from the use of the filtrate it was possible to transmit the disease further to other rabbits. The filter which they used was

described as the most permeable Berkefeld. It was fine enough, however, to hold back the microorganism of chicken cholera which was used in all filtrations as a check. Other Berkefeld filters as well as the Chamberland did not allow the virus of rabies to pass through.

Schüder, in a note opposing the idea that the protozoan described by Negri may be the cause of rabies, announces that in experiments which he conducted the virus of rabies passed through a filter which was fine enough to withhold the spirillum of Asiatic cholera, and that he was able to reproduce the disease as well with the filtrate as with the unfiltered virus.

Celli and Blasi, by the use of a Buchner press, separated the fluid from the brains of rabid dogs and infected rabbits. The fluid obtained in this way was passed through the Berkefeld filters and the filtrate was capable of reproducing the disease. Of twenty-three inoculated rabbits only three survived, and of four dogs none survived.

Remlinger, in a very recent communication, reviews the previously quoted experiments by himself and Riffat Bey and reports other experiments confirming the earlier work. The statement is made that the virus of rabies will only traverse the most porous Berkefeld (V), the other grades (N and W), together with the Chamberland filters, never having allowed it to pass. It seems probable that this virus is about at the limit, in size, of filterable microorganisms, and that normally the virus may be within certain body cells, as Nicolle and Adil Bey believe to be the case with Rinderpest.

EPITHELIOMA CONTAGIOSUM.

Quite recently Marx and Stickler have described certain experiments with a disease of birds known as "epithelioma contagiosum," which are of the greatest interest because of the bearing they may have upon the malignant tumors of man, which present just now one of the most difficult problems that experimental medicine has to deal with. This disease, known also in Germany as "Geflügelpocke," is a skin disease of chickens, geese, pigeons, and other fowls. It affects the unfeathered portions of the skin, and is very contagious.

When transmitting the disease experimentally the authors usually chose the comb or gill flaps as the point of inoculation. After scarification they were rubbed with tumor masses from diseased chickens, the disease being transmitted equally well whether the virus was taken from very recent nodules or from the scabs on fowls in the third week of the disease. The filtration experiments of Marx and Stickler were conducted as follows: The tumor masses were ground up fine in a mortar with physiological salt solution, and then one portion passed through a Berkefeld and one through a porcelain filter (variety not stated). When the filtrates were found to be free of bacteria the scarified

combs of the chickens were rubbed with them. It was found that the virus passed through the Berkefeld, but was retained by the porcelain filter. This epithelial disease must, in the opinion of Marx and Stickler, be classed among those which are caused by a filterable virus. What was apparently this same disease in pigeons could be communicated to chickens, but the chicken disease was not infectious for pigeons. By these experiments the highly specialized nature of some of these epithelial diseases is well brought out, and failures to transmit the sarcomata or carcinomata of man to the lower animals can hardly be of much value in proving the infectiousness or lack of infectiousness of those malignant tumors for man.

OTHER INFECTIOUS DISEASES.

In addition to the diseases which have been reviewed more or less in detail in the previous portion of this paper, there are several others of minor importance which it seems should be classed among those caused by a filterable virus.

The mosaic disease of tobacco is considered by Beijerinck to be due to a living fluid contagium (*Contagium vivum fluidum*). There seems to be some doubt, however, as to the nature of this disease, some considering it merely a physiological disturbance and not at all infectious in the true sense.

The myxomatous disease of rabbits, described by Sanarelli, probably belongs in the same class with those just described. Many have suggested that smallpox and vaccinia are due to an ultramicroscopic organism, but up to the present time this view is unsupported by experimental evidence.

Throughout this paper the terms "ultramicroscopic" and "invisible" have been frequently used. "Invisible" is certainly applicable to all of these viruses except that of pleuro-pneumonia. This invisibility may depend upon any one of several causes: (1) It may depend upon the ultramicroscopic size of the objects in question; or (2) upon the fact that they can not be successfully stained by any of our present methods, and that unstained do not interfere with the rays of light to a sufficient degree to make their outline discernible; or (3) the virus may have no form, but may be in solution, as suggested by Beijerinck. The last hypothesis seems to be excluded by the evidence quoted in the preceding pages to the effect that some viruses pass through the Berkefeld filter but are retained by the finer-pored Chamberland, and, in addition, the adoption of a theory of this kind involves the acceptance of principles entirely at variance with our common ideas concerning the lower forms of life, although the phenomena in question can be explained by and made to harmonize with our present and long-established understanding that a cell is the lowest form of life that can exist. The second hypothesis can not be disposed of so easily. There

is some evidence to show that certain bacteria—quite small, it is true, yet large enough to be seen with our ordinary microscopes—do pass through some of our so-called bacteria-proof filters. Their passage through these filters may be explained by their peculiar consistency which enables them to pass the pores that offer a complete barrier to some equally small but less plastic organisms.

Wherry described a number of filtration experiments with the bacillus of guinea-pig pneumonia, using Berkefeld cylinders No. 5 and No. 8, and the Chamberland cylinder F. His first experiment was with Berkefeld No. 5, a vacuum of about 660 mm. being used. The filtrate was collected in five lots of about 25 c. c. each and placed in an incubator; only the two last portions collected showed growth. A second experiment carried out in the same manner resulted similarly, the only difference being that the last one only of the five lots collected showed growth. The organism did not pass through the large Berkefeld No. 8 nor through the Chamberland F filters.

Wherry states that the bacillus of guinea-pig pneumonia is 0.5 μ wide and 0.7 μ long. This is the only record of a microorganism whose smallest diameter is not less than 0.5 μ having passed regularly through Berkefeld filters.

Von Esmarch, in the course of his endeavors to ascertain the existence of ultramicroscopic saprophytes, isolated a very minute vibrio, which he named *Spirillum parvum*. This organism, from 1 to 3 μ long and 0.1 to 0.3 μ wide, passed through Berkefeld, Chamberland F, and other filters. Neither Wherry nor von Esmarch mentions the very important matter of the time required for their filtrations, and von Esmarch fails, in addition, to note the degree of vacuum which he employed.

Borrel, in the course of certain filtration experiments with the virus of sheep pox, found that very minute water vibrios passed through certain bougies. He also found a minute protozoan 3 to 4 μ long and 0.25 μ broad. This organism was named *Micromonas mesnili*, and is said to be the smallest known protozoan. Borrel, however, used filters especially prepared and very much more porous than the Chamberland F, as has been previously explained. All of the organisms which pass these filters, however, are unusually small and actively motile. The size, consistency, and motility evidently all play a considerable part in determining the filterability or nonfilterability of microorganisms.

By a series of ingenious calculations, Errera has endeavored to determine the limit in size of microorganisms. To use his own query, May there not be microorganisms as much smaller than ordinary bacteria as the ordinary bacteria are smaller than our largest trees? Errera's calculations, based upon the "atomic theory" and the weight of molecules, lead him to believe that it is impossible for living forms

to exist which are less than one-fifth or one-tenth the size of *Micrococcus progrediens*. This micrococcus is the smallest known, measuring only 0.15 μ in diameter.

Abbé and Czapski state that microscopes as they are now constructed will not permit us to distinguish objects smaller than 0.1μ, the difficulty lying in the illumination of the field rather than in the magnifying power of the lenses.

Siedentopf and Zsigmondy have recently devised a new microscope which they claim has enabled them to distinguish particles which are completely invisible when the highest magnification of the ordinary microscope is employed. The essential feature in this new microscope consists in the method of illuminating the field. A powerful oblique light is thrown upon the objects in such a way that they become visible, appearing as minute luminous points. It is to be hoped that Siedentopf and Zsigmondy have brought forth a principle in the construction of microscopes which may be utilized in bacteriological investigations.

The value of the researches herein described can not be overestimated. In addition to the light which they have thrown upon the etiology of certain infectious diseases, they have taught us that there are many disease-producing microorganisms which can pass readily through ordinary porcelain and earthen filters, and that one at least (that of horse sickness) is not withheld even by the finest-pored filter known. They have also taught us that clear fluids which yield no culture, by whatever method used, are not necessarily sterile, but may be very infectious and in the highest degree dangerous to men and animals. These discoveries indicate also that the fields of bacteriological research present unexampled opportunities for original investigations, and notwithstanding the great advances made during the last two decades there are yet greater things to come.

A better knowledge of the etiology of a disease always leads to more intelligent and therefore more successful efforts to combat it. On this account we have reason to hope that these filtration experiments will not only increase our knowledge of the causes of maladies which are now so baffling, but that they will also enable us to do more toward their prevention and cure.

BIBLIOGRAPHY.

BEIJERINCK, W.

Ueber ein Contagium vivum Fluidum als Ursache der Fleckenkrankheit der Tabaksblätter. Cent. f. Bakt., Jena, 2. Abt., 5. Bd., No. 1, p. 27–33. 15. Jan., 1899.

BORREL, A.

Expériences sur la filtration du virus claveleux. Compt. rend., Soc. de biol., Paris, tome 54, no. 2, p. 59. 24 Jan., 1902.

Borrel, A.
Microbes des eaux et culture d'un protozoaire minimal. Ibid., p. 61.

Borrel, A.
Etudes sur la clavelée; sérothérapie et séroclavelisation. Ann. de l'inst. Pasteur, Paris, tome 17, no. 11, p. 732. 25 Nov., 1903.

Celli, A., & Blasi, D. de.
Ist das Wuthgift filtrirbar? Deut. med. Woch., Berl., 29. Jahrgang, No. 50, p. 945–946. 10. Dez., 1903.

Centanni, Eug.
Die Vogelpest; Beitrag zu dem durch Kerzen filtrirbaren Virus. Cent. f. Bakt., 1. Abt., Originale, 31. Bd., No. 4, p. 145–152; No. 5, p. 182–201. 14. Feb. and 27. Feb., 1902.

Dubois, Albert,
Une maladie infectieuse des poules à microbes invisibles. Comp. rend., Soc. de biol., Paris, tome 54, no. 29, p. 1162–1163. 31. Oct., 1902.

Von Esmarch, Erwin.
Ueber kleinste Bakterien und das Durchwachsen von Filtern. Cent. f. Bakt., Jena, 1. Abt., Originale, 32. Bd., No. 8–9, p. 561–569. 25. Okt., 1902.

Lode, A., & Gruber, J.
Bakteriologische Studien über die Aetiologie einer epidemischen Erkrankung der Hühner in Tirol (1901). Cent. f. Bakt., Jena, 1. Abt., 30. Bd., No. 16, p. 593–604. 7. Nov., 1901.

Loeffler & Frosch.
Berichte der Kommission zur Erforschung der Maul-und Klauenseuche bei dem Institut für Infektionskrankheiten in Berlin. Cent. f. Bakt., Jena, 1. Abt., 23. Bd., No. 9–10, p. 371–391. 10. März, 1898.

Loeffler.
Bericht der Kommission zur Erforschung der Maul-und Klauenseuche bei dem Institut für Infektionskrankheiten in Berlin. Cent. f. Bakt., Jena., 1. Abt., 24. Bd., No. 15–16, p. 569–574. 28. Okt., 1898.

McFadyean, J.
African horse-sickness. Journ. of comp. path. and ther., Edinburgh and London, v. 13, no. 1, p. 1–20. March 31, 1900.

Maggiora, Arnaldo, & Valenti, Gian L.
Ueber eine Seuche von exsudativem Typhus bei Hühnern. I. Mittheilung. Zeitschr. f. Hyg. u. Infektionskr., Leipzig, 42. Bd., 2 Hft., p. 185–243. 1903.

Marchoux, Salimbeni et Simond.
La fièvre jaune; rapport de la mission française. Ann. de l'inst. Pasteur, Paris, tome 17, No. 11, p. 665–731. 25 Nov., 1903.

Marx, E., & Stickler, Anton.
Untersuchungen über das Epithelioma contagiosum des Geflügels. Deut. med. Woch., Berlin, 28. Jahrgang, No. 50, p. 893–895. 11. Dez., 1902.

Nocard et Roux.
Le microbe de la péripneumonie. Ann. de l'inst. Pasteur, Paris, tome 12, no. 4, p. 240–262. 25 Avril, 1898.

Nocard, E.
La "Horse-sickness" ou "maladie des chevaux" de l'Afrique du sud. Bull. de la Soc. cent. de méd. vét., [8 sér., tome 8, no. 2] n. s., tome 19, p. 37–51. 30 Jan., 1901.

REED & CARROLL.
The etiology of yellow fever. A supplemental note. Amer. med., Phila., v. 3, no. 8, p. 301–305. Feb. 22, 1902.

REMLINGER.
Le passage du virus rabique à travers les filtres. Ann. de l'inst. Pasteur, tome 17, no. 12, p. 834–849. 25 Dec., 1903.

REMLINGER & RIFFAT-BEY.
Le virus rabique traverse la bougie Berkefeld. Compt. rend., Soc. de biol., Paris, tome 55, no. 21, p. 730–731. 19 Juin, 1903.

REMLINGER & RIFFAT-BEY.
Sur la perméabilité de la bougie Berkefeld au virus rabique. Compt. rend., Soc. de biol., Paris, tome 55, no. 25, p. 974–976. 17 Juillet, 1903.

ROUX & NOCARD.
Le microbe de la péripneumonie. Cinquantenaire de la société de biologie; volume jubilaire. Paris, 1849–1899, p. 440–449.

SANARELLI, G.
Das myxomatogene Virus; Beitrag zum Studium der Krankheitserreger ausserhalb des Sichtbaren. Cent. f. Bakt., 1. Abt., 23. Bd., No. 20, p. 865–873. 1. Juni, 1898.

SCHÜDER.
Der Negri'sche Erreger der Tollwuth. Deut. med. Woch., 29. Jahrgang, No. 39, p. 700. 24 Sept., 1903.

SIEDENTOPF, H. & ZSIGMONDY, R.
Über sichtbarmachung und Grössenbestimmung ultramikroskopischer Teilchen, mit besonderer Anwendung auf Goldrubingläser. Annalen der Physik., Leipzig, 10 Bd., No. 1, p. 1–39. 1903.

WHERRY, WM. B.
Experiments on the permeability of the Berkefeld filter and the Pasteur Chamberland bougie to bacteria of small size. Journ. of med. research, v. 8, no. 2, p. 322–328. Nov., 1902.